COMPLETE SEX-REVERSAL IN THE VIVIPAROUS TELEOST *XIPHOPHORUS HELLERI*.

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I. Introduction.

During the progress of the study on sex-differentiation of *Xiphophorus helleri* it became apparent that sex was not a fixed factor in this species. It was found (Essenberg, 1923) that 50 per cent. of the young females transformed to males before sexual maturity was reached. There were also indications that sex-reversal is not confined to immature fishes but that it may occur in adult specimens which have previously born young. To test the merits of such indications experimental plans were conceived which, together with the results, are considered in the present paper.

With the most favorable environment which prevailed at the Hull Zoölogical Laboratory and the abundance of *Xiphophorus helleri*, it was relatively an easy matter to initiate experiments. A large number of adult females were isolated into separate aquaria for observation. Close watch of these specimens was kept for more than a year but with totally negative results.

In the fall of 1922, the writer became associated with the Medical School of the University of Missouri, where the work was to be continued. Early in the spring of the following year a shipment of fish was received from the University of Chicago, for which I wish to express my gratitude to Dr. A. W. Bellamy, and another shipment was procured from the Crescent Fish Farm of New Orleans. Fishes of both shipments were mixed before they were isolated into separate aquaria. Strict records of each isolated fish were kept. The records were to show the date of isolation, date of birth of young, and the number of young at each birth.

At the beginning of July, 1923, one of the adult females which

had given birth to forty young on the second of June and fifty-three on the fourth of May failed to bring forth young. It is necessary to state here that the species under consideration reproduces monthly with almost chronological regularity. At the time mentioned there were no definite signs of sex-reversal and it was thought that owing to some change in environment, birth of young was delayed. A month later, that is the beginning of August, signs were available as to what was going on with female B16. September the 12th, when the writer left for Norman, Oklahoma, to occupy his present position, the fish had reached advanced stages of sex-reversal.

Owing to the courtesy of Dr. G. W. Tannreuther of the Department of Zoölogy, to whom I wish to express my indebtedness, my research material reached Norman in good shape a few weeks later. Female B16 was an unmistakable male except body form by the first of December and was mated to a virgin female. On February 25, 1924, this pair gave birth to eight young, all of which were to all appearances normal. Two weeks later five of the young fish died owing to a sudden drop of temperature. The remaining three reached sexual maturity with one male and two females as a sex-ratio.

The stock was greatly increased in the spring of 1924 when additional material was procured from the Crescent Fish Farm. A larger number than ever before of adult females, not less than three years of age, were isolated for observation. Of this group three females transformed to males before the end of the year. One, female C32, transformed without previously giving birth to young and for this reason is dismissed from further consideration in this study. Female C3 gave birth to one litter and female C14 three litters of young before sex-reversal took place. Both fishes were mated to virgin females, but young were obtained only from the mating in which C3 was the male. From this mating five young were obtained, all of which reached sexual maturity. The sex-ratio was two males to three females.

As stated above, in the mating in which C14 was the male no young were obtained. The mating was repeated with other virgin females but without success. Upon killing, the autopsy revealed malformation of the sperm duct which was obstructed

at its anterior end. The testes were well formed and contained abundant spermatophores.

For convenience of description the process of sex-reversal in *Xiphophorus helleri* may be divided into three, more or less, definite stages. The first ranges from a normal female to the enlargement of the anal fin, the second includes the formation of the gonopod, and the third is concerned with the development of the "sword" in the tail fin.

II. First Stage in Sex-reversal.

The first indication of sex-reversal in these fishes is the absence of the regular monthly delivery of young. To be sure, there are other factors which inhibit reproduction such as lack of fertilization, certain temperatures, light, and composition of the water, but these are exceptions and can be eliminated in normal and well-balanced aquaria. With the failure of reproduction the observer's attention is focused on other landmarks in the history of sex-reversal, the most important of which is a characteristic black area known as the puberty spot.

The puberty spot is found in all sexually mature females and is located just above the pelvic fin. Its size varies with the age of the fish or rather with the size of the ovary. In young virgin females it is less than one fourth of an inch in diameter, whereas in females of three years of age it approaches half an inch in diameter. The puberty spot is caused by the black pigment visible through the body wall which accumulates in the peritoneum adjacent to the ovary. With onset of sex-reversal the black pigment in the peritoneum gradually fades and in the course of six to eight weeks the puberty spot has entirely disappeared.

Sex colorations in *Xiphophorus helleri* exhibit very little, if any, dimorphism. During sex-reversal the bright red and black pigments of the fins and lateral line dim to such an extent that the fish becomes, so to speak, "invisible." This appearance of the animal is retained until the gonad of the opposite sex has begun to grow and is capable of producing a certain amount of the male sex hormone.

The internal changes which take place during this stage are

significant and must, to a certain extent, be reviewed here. For further details of description and figures on sex-differentiation the reader is referred to Essenberg (1923).

As far as the writer knows, the causative factor or factors in sex-reversal have not been successfully demonstrated. That these factors reside in the gonad and possibly in the germ cells themselves has been substantiated beyond a doubt by experimental data. Whenever those factors become functional the entire ovary is subject to hopeless degeneration. At first the large ovocytes disintegrate, then the medium-sized ones, and finally the young ovocytes which are found close to the epithelium of the ovarian cavity follow their two predecessors. Also the epithelium covering the ovary, which originates from the peritoneum of the body cavity, disintegrates completely. The epithelium of the ovarian cavity, however, is not subject to disintegration. It shrinks and assumes more or less a tubular form, its cells, judging from their appearance, undoubtedly undergo retrogressive changes but no disintegration is noticeable. After a period of rest this epithelium becomes active and proliferates cells which go to form the gonad of the opposite sex—the testis. The process of testicular formation which belongs to the present stage of sex-reversal includes the proliferation of germ cells from the remains of the epithelium of the ovarian cavity and the formation of sex-cords. It includes two stages of sex-differentiation called by the writer "Early and Middle Stages of Tubule Formation" (Essenberg, 1923, pp. 57–58).

It is interesting to note that the epithelium of the ovarian cavity which gives rise to the definitive male cells originates, as does the epithelium covering the ovary, from the peritoneum of the body cavity.

While the process of disintegration is taking place, the vitality of the transforming animal is reduced to the minimum. Voluntary motion which is so characteristic of *Xiphophorus helleri* is seldom noticed; the fish rests most of the time and moves only to escape danger or because of hunger. As to the latter it may be said that the animal takes very little food of any kind during the process of ovarian disintegration. This is a critical stage to the animal. The least change in the animal's environment,

such as slight fluctuation in temperature, water composition or disturbance will inevitably result in the death of the fish. It seems probable that sex-reversal in *Xiphophorus helleri* is not preceded by the increase of metabolic rate but that the opposite is true. Nor is it true that the transformation is caused by tuberculosis or any other kind of disease as far as it can be detected either macro- or microscopically.

III. Second Stage in Sex-reversal.

The second stage in sex-reversal deals with the transformation of the anal fin of the female into a functional intromittent organ or gonopod and with the parallel development of the gonad.

The anal fin of the female consists of ten pairs of rays all of which are approximately of the same length and diameter. The above statement applies equally well to the anal fin of young fishes during the indifferent stage of development. During sex-differentiation in the male direction or sex-reversal, which in *Xiphophorus helleri* is always from female to male, several pairs of the rays undergo a peculiar metamorphosis to form a copulatory organ. The first sign of such transformation is the thickening of the third pair of rays. While this is in progress, the third, fourth, and fifth pairs of rays elongate until approximately twice the length of the original fin is reached. The first, second, and sixth to tenth pairs of rays are subject to no special changes and remain rudimentary. The third pair reaches a thickness several times its original diameter and serves as a supporting bar or rod of the gonopod. The apex of this pair of rays together with the fourth and fifth pairs form hooks and counterhooks which serve the purpose of anchorage to the female genital pore during copulation. It is not a hollow penis-like structure but a solid bar which acts as a guide in transmitting of spermatophores from male to female genitals.

The development of the gonad during the second stage of sex-reversal corresponds to the "Late Stage of Tubular Formation" in sex-differentiation. The solid sex-cords which were formed during the first stage acquire a lumen and become branched and subbranched. The cells constituting the sex-cords or tubules, which thus far were not unlike their sister cells of the

peritoneum undergo characteristic transformation. The cytoplasm as well as the nucleus increases in size and assumes a spherical form. When these cells have completed their differentiation they can not be distinguished microscopically from the primordial germ cells of the indifferent stage.

IV. The Third Stage in Sex-Reversal.

The third stage in sex-reversal is characterized externally by the development of a "sword"-like process in the tail fin and internally by spermatogenesis.

It is true that there is a slight overlapping between the second and third stages, yet it is desirable to consider these separately for the reason that they constitute definite landmarks in the process of sex-reversal.

The sword of the caudal fin begins to develop slightly before the gonopod reaches its completion. It is formed from the ventral lobe of the tail fin. The ten ventral rays of this fin are involved. Most of the elongation falls upon the sixth to the tenth and the utmost length is reached by the eighth ray. The length of the fully formed sword approximates the total length of the fish. It is abundantly supplied with brilliant colors and its sole purpose seems to be display.

The development of the testis in this stage of sex-reversal consists of spermatogenesis. A number of germ cells develop in a constricted portion of the sex-cord or tubule and form an acinus or spermatocyst. Every cell in such a cyst is in the same stage of development from the beginning to the end and they ultimately form the spermatophore. At the end of the so-called "Middle Stage of Tubular Formation," the germ cells are primary spermatocytes. The secondary spermatocyte stage is reached by cell division which doubles the number of germ cells and the volume of the acinus. With another cell division which doubles the cell number but not volume of the acinus the germ cells reach the spermatid stage. With the metamorphosis of spermatids into spermatozoa the acinus which thus far has been so tightly packed with germ cells that it simulates a syncytium now becomes luminated. The lumination owes its origin to the fact that the spermatozoa move towards the

periphery of the acinus and form a lining to the thin membranous wall of the acinus. The lining consists of only one layer and is formed by the nuclei or heads of the spermatazoa. The tails project freely into the lumen. With this the acinus has metamorphosed into the mature spermatophore which during copulation reaches the female genitals by the aid of the gonopod.

V. Discussion.

Fertility before Sex-Reversal.

To eliminate the chance of error and prevent misunderstanding, it was decided that only those females which are definitely known to have produced young will be considered in the study of sex-reversal. The records of female B16 shows that she gave birth to 53 young on the 4th of May, and to 40 young on June the 2d, 1923. The records of female C3, which are equally complete, show that only one litter of young were born before she began to change in the male direction. If it is remembered that the females of *Xiphophorus helleri* reach sexual maturity before they are one year of age, and that the fishes were at least three years of age before they came under the writer's observation, it is seen that females B16 and C3 have been experiencing the instincts of motherhood not less than two years before they experienced the instincts of fatherhood.

Fertility after Sex-Reversal.

From the mating in which B16 was the male, eight young were born. C3 became the father of five young, all of which developed to normal, sexually mature fish. It can be stated that the mortality in the progeny of B16 was wholly due to the inability to control temperature in the aquaria. The sex ratio of the young obtained compares favorably with sex ratios found by the writer in his study of sex-differentiation (1923).

The Origin of Testicular Tissues.

It has been shown (Essenberg, 1923) that the definitive germ cells of *Xiphophorus helleri* are not linear descendants of primordial germ cells but originate from peritoneal epithelium. The primordial germ cells either entirely disintegrate (females) or

become segregated and thus never take part in definitive germ cell production (males). The epithelium of the ovarian cavity which gives rise to definitive ovocytes proliferates cells which go to form the testes of the transforming fish. The epithelium of the ovarian cavity originates in the following fashion. As the primordial germ cells of this teleost project into the body cavity, they are covered by the peritoneal epithelium. During the indifferent stage the gonads are paired. If the young fish develop in the male direction, the paired or bilateral relation is retained permanently. In case the animal develops into a female, the undifferentiated bilateral gonads fuse medially in such a manner that the medial parts of the covering of the gonads form the epithelium of the ovarian cavity. In the course of sex-reversal the entire ovary disintegrates except the epithelium of the ovarian cavity. From it cells proliferate which form sex cords and later seminiferous tubules.

Proof to the effect that the same is true in birds was furnished by the excellent work of Fell (1923). This author had the unique opportunity to study the gonads of eight arrhenoid birds which represented the various stages in sex-reversal from female to male. It was found that the germ cells of the opposite sex proliferate from the peritoneal epithelium of the degenerating ovary as well as from the peritoneal epithelium of the vestigial right ovary. These cells form sex cords which in due time give rise to seminiferous tubules. The sperm cells from such tubules are known to be functional. The author concludes that "we have here a case of direct transition of ordinary somatic peritoneal cells into germ cells."

Similar observations have been made by Gatenby (1916), Firket (1920), and by Swingle (1921). In the light of our present knowledge, it may indeed be questioned if definitive germ cells ever are lineal descendants of primordial germ cells in higher animals.

It has been claimed that the peritoneal epithelium covering the ovary is "packed" with primordial germ cells which have reached the epithelium by migration and that those are the primordia of sex cord formation. Whatever might be the validity of this claim in case of birds the writer is not in position

to say, but merely wishes to point out that the claim is inapplicable in case of *Xiphophorus helleri*. The primordial germ cells reach the body cavity en masse by passive displacement brought about by developmental processes of adjacent musculature and at no time do germ cells fuse with cells of peritoneal epithelium.

Causes of Sex-Reversal.

A case of complete sex-reversal in ring dove was described by Riddle (1923). The autopsy revealed that the viscera of the bird were seriously affected by tuberculosis which, together with other cases observed by Riddle, led him to the conclusion that tuberculosis is the immediate cause of sex-reversal. Based on the fact that the male bird has a higher metabolic rate than the female, Riddle assumes that the function of tuberculosis is to increase the metabolic rate of the diseased female bird. When once the metabolic rate has been increased, the diseased animal, according to Riddle, has nothing to prevent it from acquiring the anatomy and physiology of the opposite sex.

The data gathered from fishes undergoing sex-reversal do not seem to favor the claim that there is an increase of metabolic rate prior to, or during, transformation of sex. Indeed there are definite indications that the opposite is true. The transforming fishes become very inactive, probably take no food during early stages and are incapable of adapting themselves to changing environment even if the change be very slight.

Another case of complete sex-reversal in birds was describe by Crew (1923). This bird, a Buff Orpington hen, was als affected with tuberculosis of the viscera. Crew claims that sex-reversal is preceded by some disease such as tuberculosis, hemorrhage, tumor growth, etc. The disease affects the ovarian secretion and the general metabolism of the bird in such a way that the conditions favorable for the differentiation and growth of spermatic tissue are created.

It is definitely known that the fishes described in this paper were not diseased, certainly not by tuberculosis. Every transformed fish has been sacrificed to establish this very point. A number of diseased fishes have been observed, but none of these have become subject to sex-reversal. A case of complete

sex-reversal in the domestic fowl is described by the writer (1926) in which no disease whatever is discovered. From the above fact the conclusion is warranted that disease is not a necessary precursor of sex-reversal.

It seems unquestionably true that the factor or factors which determine and control sex must also determine and control sex-reversal. It seems equally true that such factor or factors are of labile rather than fixed nature.

The chromosome theory has been considered as an adequate explanation of sex determination and control. It is assumed that sex is determined at fertilization at which the zygote receives the male or female constitution which it maintains for life.

In the light of data accumulated for such studies as hermaphrodites, intersexes and particularly from cases of complex sex-reversal, the chromosome theory seems to have reached the limit of flexibility. It is difficult to see how a definite chromosome composition which determines and controls sex can be completely overridden and still remain as a sex-controlling mechanism. The inadequacy of the chromosome theory finds expression in the often reiterated statement that "sex is not irrevocably decided by the sex-chromosome constitution." It seems logical to expect that there must be something that does decide sex and possibly the 'sex-chromosomes.'

Proof that maleness and femaleness depends upon the secretions of the testes and ovary has been supplied by a number of investigators. Perhaps the clearest cut data have come from castration and transplantation experiments, particularly from the work of Zawadowsky (1922). This author finds that after a total castration of a young cockeral he loses his voice, sex instincts, and head-furnishings and thus assumes an asexual or generic appearance. However, if an ovary is successfully implanted into such castrated male, it will not only assume the appearance of the hen but the graft produces ovocytes as well. In case a young hen is castrated, she will acquire cock-feathers and spurs after the first molting. If however, the castration is incomplete, and the ovary has the chance to regenerate, the bird regains its female furnishings. Hens with total removal of ovary, develop testes as well as external fur-

nishings of the male within four to five months. Castrated hens with implanted testes acquire male furnishings and if the transplantation is successful, the graft produces active spermatozoa.

From these and other experiments of similar nature which he has carried on with mammals, Zawadowsky concludes as follows: "Aus meinen Versuchen bin ich geneigt Hinweise darauf zu ersehen, dass hinter den Symbolen der 'Geschlechtsgene' F (female) und f (male) die geschlechts hormonen- Feminisin und Masculinisin zu suchen sind."

With the above statement the present writer is in full accord and wishes to add that with the replacement or substitution, if you please, of feminisin or the female hormone for the gene-anatomy represented by X and masculinisin or male hormone for the qualities of Y, the problems of sex are brought in accord with present knowledge of bio-chemistry and endocrinology. By so doing we not only gain an insight into the mechanics of the various types of hermaphrodite, of "intersex," and sex-reversal cases, which thus far have been a source of puzzle and speculation, but we also gain a better understanding of the science of genetics.

In conclusion, it may be said that there is no particular disease associated with sex-reversal. Any adverse condition be it extrinsic or intrinsic, acquired or inherited, which tends to reduce the capacity for hormone production beyond a certain limit may automatically become the immediate cause in sex-reversal in the female of *Xiphophorus helleri*.

VI. Summary.

1. Two cases of complete sex-reversal, from female to male, in adult *Xiphophorus helleri* have been described.

2. It is definitely known that both fish gave birth to normal young prior to sex-reversal.

3. It is also definitely known that both fish fertilized virgin females which gave birth to young with sex-ratio typical of the species.

4. The transformed fish is indistinguishable from a "normal" male except body form which is that of a female.

5. The entire ovary disintegrates except the epithelium of the

ovarian cavity. From it cells proliferate and form sex cords in sex-reversal cases.

6. The epithelium of the ovarian cavity is derived from the peritoneal epithelium and at no time is infiltrated with primordial germ cells.

7. The position, structure and physiology of the testis of the transformed fish is identical to that of the "normal" males. The oviduct becomes the spermduct.

8. No tubercular lesions or lesions of any other disease were found in the arrhenoid fishes.

9. Chromosomes are not considered as having the function of sex determination and control.

10. Sex is determined and controlled by the sex hormones derived from the ovary and testes.

11. Any agent or condition which tends to decrease the capacity for hormone secretion becomes an immediate factor in sex-reversal.

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